IMPROVED CULTURE TECHNIQUES FOR MASS REARING *GALLERIA MELLONELLA* (LEPIDOPTERA: PYRALIDAE)[1]

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ABSTRACT: Supplementing an artificial diet with 5% pollen, honey, or beeswax significantly increased survival of adult *Galleria mellonella*. Survival on the basic diet averaged 27.4%. When honey, pollen, or wax was added, survival was 44.7%, 80.8%, and 89.6%, respectively. Developmental time for moths fed diets containing 5% pollen or a combination of pollen, honey, and wax was shorter by approximately 2-5 days. Newly emerged virgin females which had fed as larvae on a 100% pollen, honey, and wax diet weighed 36% more than females derived from larvae fed the artifical diet. A phagostimulatory hypothesis is suggested.

Additionally, 78% of prepupae accepted cut plastic soda straws as puparial sites and spun their cocoons in them. This greatly facilitated the handling of individual prepupae and pupae and should be a convenience to both research programs and commercial wax moth producers.

The greater wax moth, *Galleria mellonella*(L.), is easily reared and is often used as a laboratory animal. However, our initial attempts to establish a culture from wild stock resulted in low survival rates (ca. 30%) when an artificial diet [Stoneville(SV) developed by King *et al.* 1979] was used. However, pilot studies indicated that survival was good (ca. 95%) on a diet composed wholly of their naturally occurring larval food, i.e. pollen, honey, and beeswax. Inspection of the artificial diet (King *et al.* 1979) did not reveal nutritional shortcomings. Previous studies have shown that small amounts of beeswax improved growth rates (Beck 1960, Dadd 1966, Young 1964). Further, Dadd (1966) observed higher survival rates during portions of larval development when beeswax was added to an artificial diet. These findings suggested that small amounts of natural food could play an important nutritional role in adult survival. We report here the results of a study done to determine why survival differed so strikingly on artificial diet compared with a diet composed of natural ingredients.

MATERIALS AND METHODS

Six diets were tested. Their composition by weight was as follows: 1) 100% Stoneville(SV), 2) 95% SV + 5% pollen(P), 3) 95% SV + 5% honey(H), 4) 95% SV + 5% beeswax(W), 5) 95% SV + 5% PHW, 6) 100% PHW.

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The Stoneville diet consists largely of baby foods (Gerber® mixed and high protein cereals), gycerol, wheat germ, water, sucrose, and vitamins (see King *et at.* 1979 for details). Its protein composition is about 13%. The pollen-honey-wax diet was made by mixing bee-collected pollen (63%; see Dietz 1982 for plant species represented) with chipped honey comb (37%). The honeycomb (cappings) contained approximately 50% honey. The protein content of this diet is estimated to be about 13%. Twenty grams of diet were placed in 100ml glass rearing jars, sealed and kept frozen until used. Fortified diets were prepared by placing 19g of SV diet in the rearing jar, and then 1g of either pollen, honey, or wax was sprinkled or dripped onto its surface.

Moths were obtained by removing overwintering prepupae from a dead honey bee hive in Athens, Georgia. They were incubated at 30°C. Adults emerged in about two weeks and mated *inter se*. Eggs were collected between strips of pleated wax paper. Three days after laying, egg masses were divided into small clusters of 10-15 eggs. These clusters were placed on filter paper in petri dishes and incubated at 30°C + 2°C. Just before hatching, eggs were examined and clusters containing at least 10 larvae that appeared normal were selected for testing. Excess larvae were destroyed by puncturing the eggs with a needle. Eggs were then placed in small aluminum foil baskets (16mm x 10mm) and randomly assigned to diets. Twenty replications of each diet were performed. After addition of the eggs, the rearing jars were sealed with a solid metal screw lid containing a rubber interseal. Rearing jars were held at 32°C ∓ 1°C, 40 ∓ 10% RH, and 12:12LD. Seven days after infestation, egg clusters were removed and per cent hatch determined. Also, the solid lids were replaced with a similar lid containing a 9mm hole. This hole was covered with a piece of transparent tape, sticky side out. Several small holes were punched in the tape with a small pin to allow air exchange. When the first prepupae were observed, this tape was replaced with a circular piece of aluminum screen. At this stage, rearing jars were inspected daily. As adults appeared they were removed, sexed, and their emergence date noted. The first twenty adult virgin females that emerged from the SV and the pollen-honey-wax diets were weighed. Sixty days from the time of egg laying, the contents of the rearing jars were examined. Cocoons were counted and pupal mortality noted.

Survival and emergence data were analyzed with a 1-way ANOVA, and differences among treatments evaluated with Duncan's multiple range test. Student's t-test was used to determine differences in adult female weights.

To confirm that the supplemented Stoneville diet was suitable for a mass rearing program, we fed the Stoneville+5% fresh frozen pollen to ca. 6,000 hatching *G. mellonella* larvae (eggs were weighed, not counted).

As prepupae emerged from the diet and began spinning light cocoons, they were removed and placed in 3.81 glass jars containing cut plastic soda straws (2 X 0.5cm; Sweetheart[R]). About 70 prepupae were added to jars containing 200-300% excess of cut straws (scattered horizonally on the bottom of the jar), which were covered with a single circular piece of toweling. Jars were kept in a darkened incubator (same conditions as in above dietary trials).

RESULTS

The addition of 5% of either pollen, honey, or beeswax (PHW) to the Stoneville(SV) diet resulted in a significant increase in adult survival (Table 1). Adult survival on the basic diet averaged 27.4%. The addition of honey increased survival to 44.7% ($P < 0.05$). Fortification with either pollen, wax, or a combination of pollen, honey, and wax produced survival rates that did not differ significantly from the control diet (89.2%).

Males reared on the SV+5% pollen, SV+PHW, and control diet eclosed in a significantly shorter time (ca. 3-5 days, $P < 0.05$) than moths on the other three diets (Table 1). Average time to first emergence was slightly shorter for males (0.2-1.3 days) than that required by females, but this difference was not significant. Newly emerged virgin females weighed on average less when reared on the SV diet, than on the control diet (114 and 156mg, respectively; $P < 0.001$). During the mass rearing trial 78% of prepupae spun their cocoon inside the cut soda straws. The adult survival rate for larvae fed the Stoneville+5% pollen diet during this trial was estimated to be about 84%.

DISCUSSION

The striking increases in survival caused by small amounts of pollen, honey, or beeswax was not the result of an altered physical consistency, nor the addition of essential nutrients (Haydak 1936, 1940, Allegret 1964; Dadd 1966; Marston and Campbell 1973). Dadd (1966) suggested that the inclusion of beeswax served as a source of metabolic water. However, the basic Stoneville diet contains about 17% water and supports good larval growth (King *et al.* 1979). The 300% increase in survival with the addition of 5% beeswax to this diet suggests that beeswax performed an additional function.

Eischen *et al.* (unpublished) found in a preliminary test that newly hatched larvae preferentially chose artificial diets to which alcoholic extract of pollen was added. This suggests that pollen provided phagostimuli attractive to *G. mellonella*. Other observations support this view. Balazs (1958) reported that newly hatched larvae preferred honeycomb to an artificial diet, even though this diet contained honey and beeswax. He also noted that older larvae fed the same artificial diet chose honeycomb when given the opportunity. Haydak (1936) reported that larvae attacked old combs in those areas that contained pollen. We have on numerous occasions observed newly hatched larvae that have been given the Stoneville diet wander about the inner walls of their rearing container when food was nearby. Newly hatched larvae given a honey-pollen-wax diet were rarely seen away from the food. The natural history of this moth also lends support to a phagostimulatory hypothesis. Adult females oviposit in and around bee colonies; generally in crevices where eggs are protected from the bees (Paddock 1918, Nielsen and Brister 1977). Once hatched, the larvae must search for food. Though distances may be short, it would be adaptive to be able to locate food (which also serves as shelter) rapidly in the hostile interior of a honey bee colony.

The shorter times to eclosion of moths fed diets containing pollen indicate that even small amounts of pollen are effective in promoting development. Since pollen was localized on the upper surface of the diet mass, it seems unlikely that its consumption would have been uniformly distributed during larval growth. If the phagostimulatory hypothesis is true, then consumption and consequent beneficial effects occured during the earliest instars. From this it follows that an early pollen meal may supply a nutrient, perhaps protein, that allows faster development, while apparently wax and honey do not.

Prepupal acceptance of soda straws greatly facilitated the handling of prepupae and pupae. Large numbers of known age groups can be held in relatively small containers. This is convenient when storing prepupae under refrigerated conditions (ca. 15.5°C). We find that chilled, lightly spun prepupae are easy to use by fishermen, a slight squeeze on the straw prompts the prepupae to crawl out. Because male prepupae emerged from the diet slightly before females, the first harvest of prepupae results in a high percentage of males. This is an added benefit when separation of sexes is desired. Since these tests, we have successfully reared many thousand *G. mellonella* using this technique.

These findings should be of value to those who wish to preserve the genetic variation observed in wild stock or to increase the yield of *G. mellonella* in newly-established cultures (Bush 1975). However, cultures that have been reared for many generations on a particular artificial diet and consequently undergone selection for it may not show greatly improved survival. Nevertheless, under some conditions it could be prudent as well as cost effective to supplement artificial wax moth diets with small amounts of properly stored pollen or unprocessed honeycomb.

Table 1. Developmental characteristics of *Galleria mellonella* reared on an artificial diet fortified with honey, pollen, or beeswax

Diet	% adult survival ($\overline{X}$±SE)	days to 1st ♂ emergence ($\overline{X}$±SE)	days to 1st ♀ emergence ($\overline{X}$±SE)	adult ♀ weight ($\overline{X}$±SE)
Stoneville	27.4 ± 4.7 a[1]	42.7 ± 1.1 a	43.9 ± 0.8 a	114.2 ± 5.3 mg[2]
Stoneville + 5% honey	44.7 ± 6.0 b	43.3 ± 1.3 a	43.5 ± 0.8 a	---
Stoneville + 5% wax	89.6 ± 3.8 c	42.8 ± 0.7 a	43.7 ± 1.0 a	---
Stoneville + 5% pollen	80.8 ± 4.1 c	39.7 ± 0.8 b	41.0 ± 0.9 b	---
Stoneville + 5% honey, pollen, wax	82.5 ± 4.7 c	39.2 ± 0.8 b	39.4 ± 0.8 c	---
Honey, pollen, wax	89.2 ± 2.6 c	37.6 ± 0.7 b	37.8 ± 0.7 cd	156.0 ± 5.8

[1]Means in the same column followed by the same letter do not differ significantly at the 5% level according to Duncan's multiple range test.

[2]Means weights are significantly different ($P < 0.001$). Female weights on other diets were not recorded.

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ANNOUNCEMENT

July 31-Aug. 3, 1990. International Symposium on Biotic Stresses of Barley in Arid and Semi-Arid Environments. Huntley Lodge, Big Sky, Montana. Contact: Jack Riesselman, Dept of Plant Pathology, Montana State University, Bozeman, MT 59717. Phone: 406-994-5149 FAX: 406-994-6579.